

More precisely described, the invention is an automated method for detecting the presence of oxidants in a urine sample comprising placing an aliquot of the urine in a first automated analyzer sample cup, placing a standard of known concentration of oxidant in a second automated analyzer sample cup, placing the cups in a sample tray within the automated analyzer, transferring the urine from the first sample cup and the standard from the second sample cup to discrete cuvettes mounted within the automated analyzer, injecting an aqueous reagent composition comprising an acid at 0.01 N concentration or higher and one or more phenylamine chromogenic indicators from the group consisting of

N,N,N',N'-tetramethyl-1,4-phenylenediamine, N,N-diethyl-1,4-phenylenediamine,

2,3,5,6-tetramethyl-1,4-phenylenediamine, N,N-dimethyl-p-phenylenediamine,

2,4,6-trimethyl-1,3-phenylenediamine, N,N,N',N'-tetramethylbenzidine,

3,3,5,5-tetramethylbenzidine, N,N,N',N'-tetramethyl-4,4-diaminestilbene and O-tolidine.

into cuvettes and mixing and determining the sample-reagent mixture's absorbance with the automated analyzer's spectrophotometer at a preprogrammed wavelength between 400 and 700 nm at a preprogrammed time between 12 seconds and 600 seconds after the phenylamine chromogenic indicator is added to the cuvettes and comparing the absorbance of the urine-reagent mixture to the absorbance of the standard-reagent mixture and thereby determining if the sample has an abnormal amount of oxidant compound present. The reagent may also contain potassium iodide to enhance sensitivity to bleach and iodine containing oxidants. This enhanced sensitivity is important because bleach and some other halides will bind to nitrogen containing compounds in urine.

Nitrogenous compounds are typically found in urine and include urea, uric acid, and proteins. Bleach in particular will bind to these compounds very quickly (less than 4 hours). Potassium iodide greatly enhances the reactivity of bound bleach with phenylamines.

This oxidant-detecting reagent is placed in the auto-analyzer along with samples and standards to be analyzed. The instrument aliquots samples into individual cuvettes, adds reagent, and measures the absorbance of each test sample at a specific wavelength and compares these absorbance readings to that of a known standard to determine the presence or absence of oxidants.

More precisely described, the invention is an automated method for detecting the presence of oxidants in a urine sample comprising placing an aliquot of the urine in a first automated analyzer sample cup, placing a standard of known concentration of oxidant in a second automated analyzer sample cup, placing the cups in a sample tray within the automated analyzer, transferring the urine from the first sample cup and the standard from the second sample cup to discrete cuvettes mounted within the automated analyzer, injecting a first aqueous reagent composition comprising potassium iodide and one or more buffering compounds into the cuvettes, injecting a second aqueous reagent composition comprising an acid at 0.01 N concentration or higher and one or more phenylamine chromogenic indicators from the group consisting of N,N,N',N'-tetramethyl-1,4-phenylenediamine,

N,N-diethyl-1,4-phenylenediamine, 2,3,5,6-tetramethyl-1,4-phenylenediamine,

N,N-dimethyl-p-phenylenediamine, 2,4,6-trimethyl-1,3-phenylenediamine,

N,N,N',N'-tetramethylbenzidine, 3,3',5,5'-tetramethylbenzidine,

N,N,N',N'-tetramethyl-4,4'-diaminostilbene and O-tolidine into the cuvettes and mixing

and determining the sample-reagent mixture's absorbance with the automated analyzer's spectrophotometer at a preprogrammed wavelength between 400 and 700 nm at a preprogrammed time between 12 seconds and 600 seconds after the phenylamine chromogenic indicator is added to the cuvettes and, comparing the absorbance of the urine-reagent mixture to the absorbance of the standard-reagent mixture and thereby determining if the sample has an abnormal amount of oxidant compound present.

EXAMPLE IV

Prepare a solution containing:

R1

- a) 11.75 g Potassium Iodide
- b) 34.0 g Sodium Acetate
- c) 2.94 mLs 5.0 N Sodium Hydroxide
- d) QS to 1 liter with water.

R2

- a) 0.1 g DEPD (Diethyl-1,4-phenylenediamine sulfate)
- b) 0.333 g N,N,N',N'-Tetramethyl-1,4-phenylenediaminedihydrochloride
- c) 6.9 mLs Phosphoric Acid
- d) QS to 1 liter with water.

This formulation is added to samples at a ratio of 1 to 7 to 7 (e.g. 18 μ L to 130 μ L to 130 μ L). This assay would be calibrated with 150 mg/L of nitrite standard and absorbance measured at 570 nm. This formula has good sensitivity to bleach, nitrite, chromate, iodate/iodic acid, and peroxide/peroxidase. The potassium iodide acts to intensify the reactivity of bleach and iodic acid.

Typical Hitachi 717 parameters for the Oxidant Assay:

ASSAY CODE	1 POINT [37] - [0]
SAMPLE VOLUME	[18]
R1 VOLUME	[130] [100] [NO]
R2 VOLUME	[130] [100] [NO]
WAVE LENGTH	[] [570]
CALIBRATOR METHOD	[LINEAR] [0] [0]
STD (1) CONC. POS	[0] - [-]
STD (2) CONC. POS	[150] - [-]
UNITS	[mg/dL]
SD LIMIT	[999]
DUPLICATE LIMIT	[32000]
SENSITIVITY LIMIT	[0]
ABS. LIMIT	[32000] [INCREASE]
PROZONE LIMIT	[0] [UPPER]
EXPECTED VALUE	[0] - [1000]
TECH LIMIT	[0] - [1000]
INSTRUMENT FACTOR	[1.0]

EXAMPLE V

Prepare a liter of solution containing:

A. 0.25 g N,N,N',N'-tetramethylbenzidine

B. 50 mLs 5 N Hydrochloric Acid

C. QS with water to make 1 liter.

This formulation is added to samples at a ratio of 13 to 1 (e.g., 15 μ L sample to 200 μ L reagent).

This assay would be calibrated with nitrite as the standard (200 mg/mL Nitrite) and absorbance measured at 415 nm. This formulation has good sensitivity to nitrites, chromate, and peroxide/peroxidase, but not to low levels of bleach and iodic acid. One could include 1.0 mL of Brij-30 per liter of reagent.